Molecular modelling of conformational changes in solvated α_{s1}-casein peptides

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Abstract

Computer models of α_{s1}-casein peptides were simulated in an aqueous environment to compare their conformations as separate peptides with structural features of corresponding regions in the intact α_{s1}-casein molecular model. Peptide sequences selected for investigation were based on sites of potential cleavage by chymosin and/or plasmin. Four large peptides and several smaller peptide fragments were refined by applying a series of molecular dynamics simulations and energy minimizations. Most peptides adopted conformations that were more compact than the conformations of the corresponding sequences in the intact α_{s1}-casein model. The results indicated that sequences in the peptides that promote the open, flexible structure of the parent protein are likely to resist formation of new secondary structure during refinement. However, these regions can develop more local flexibility and additional turns because of the high proline content of α_{s1}-casein. The region in which seven of the eight phosphoserine residues are located maintained its conformation throughout the refinement, but this region adopted a new conformation when phosphoserines were replaced with serines. The results demonstrate the potential for folding in casein peptides and highlight the features that promote the open, extended structures of the caseins. Published by Elsevier Science Ltd.

Keywords: α_{s1}-Casein; Peptides; Molecular modelling

1. Introduction

Caseins are found in the milk of all mammalian species. Characterized by a high content of proline (11%) and very low content of cysteine (0.5%) (Eigel et al., 1984), they have open, extended, and flexible conformations that provide solvent accessibility and facilitate binding of calcium ions and colloidal calcium phosphate. Despite the limited presence of much α-helix, caseins have a secondary and tertiary structure with as much as 30% in extended, or β-sheet-like, conformations and a similar percentage of turns (Kumosinski, Brown & Farrell Jr., 1993a, b, 1994a). Comparison of amino acid sequences from different species suggests only moderate sequence homology (Holt & Sawyer, 1988, 1993), but caseins contain many residues that are invariant (same residue at each position) or conserved (substituted by an amino acid of similar ionic, hydrophobic, or polar character). The totals of invariant plus conserved residues are: α_{s1}-casein, 37%; α_{s2}-casein, 11%; β-casein, 30%; κ-casein, 31%.

An important characteristic of caseins is that they cannot be crystallized, a feature that demands ingenuity and insight in investigations of casein structure. Although solution studies and ultrastructural analysis have yielded much information, sequence-based molecular modelling of caseins has provided the first detailed representations of casein conformations (Kumosinski et al., 1993a, b, 1994a; Kumosinski, King & Farrell, 1994b). The success of that research suggests that more detailed information might be developed by applying molecular modelling techniques to casein fragments or peptides. The conformations that combine to produce the open, extended structure of α_{s1}-casein could then be dissected into those that arise from sequence alone and those that are influenced by neighbouring strands of the folded protein.

Previously, we used molecular modelling techniques to examine the folding of α_{s1}-casein peptides (Malin & Brown, 1995). The results suggested the basis for the reorganization of electron density in cheese during

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storage (Cooke, Tunick, Malin, Smith & Holsinger, 1995; Tunick, Cooke, Malin, Smith & Holsinger, 1997) and the correlation of α_{s1}-casein breakdown with improvement in cheese texture and meltability in low-fat Mozzarella cheese (Malin, Tunick, Smith, Shieh, Sullivan & Holsinger, 1994; Tunick et al., 1993). However, those studies were conducted on structures modelled in vacuo and do not necessarily reflect conformations in the aqueous systems of milk and dairy products.

Molecular modelling of water-solvated peptides is now possible with new software programs, and the work reported here focuses on the structural features that α_{s1}-casein peptides adopt in reaching stable, energetically favourable conformations in the solvated state. It must be emphasized that this research is completely theoretical and was developed to demonstrate the potential of casein peptide conformations to change, partially or completely, as α_{s1}-casein undergoes proteolysis, as in cheese. Further, in real systems the final structure of any peptide will be strongly influenced by neighbouring caseins and other peptides. The studies presented here give an overall view of the potential for peptide folding in α_{s1}-casein fragments, show the limitations to folding imposed by a peptide sequence, and demonstrate the features that provide casein with its unique structure.

2. Experimental

Peptides selected for study were based on sites of chymosin and/or plasmin cleavage in α_{s1}-casein reported by McSweeney, Olson, Fox, Healy and Højrup (1993a,b) and were simulated with the molecular modelling program SYBYL, Version 6.3 (Tripos Associates,[1] St. Louis, MO, USA). Each peptide was created using a subroutine that excised the corresponding sequence with its conformation from the unsolvated model of α_{s1}-casein (Kumosinski et al., 1994a), which was refined in vacuo. The excised sequence was 'blocked' by adding an *N*-methyl blocking group (NME) to the carboxyl terminal and an acetyl group (ACE) to the amino terminal, after which the blocked peptide was solvated using the SYBYL droplet algorithm. Refinement then proceeded through a series of molecular dynamics simulations, at 50° or 100°K, followed each time by energy minimizations, until the total energy of the system was at least -5 to -10 kcal per residue per mole. Kollman charges (Weiner, Kollman, Nguyen & Case, 1986) and the Tripos force field (Clark, Cramer III & Van Opdenbosch, 1989) were utilized for energy minimizations and dynamics. The general procedure is similar to that described earlier (Malin & Brown, 1995), except that a solvation step takes place before simulations begin.

Much of the energy input during molecular dynamics simulation of a solvated peptide is absorbed by surrounding water molecules, rather than the peptide sequence. As a result, more molecular dynamics operations are required, and for longer duration, than for similar operations in vacuo. Therefore, hydration levels for each peptide were kept to approximately 0.5–1.0 g of water/g of protein to minimize computer time while still maintaining levels of bound water within the range of those observed in globular proteins and in caseins. Analytical subroutines of SYBYL were used to compare structural features of the peptides in their initial and final states.

3. Results

3.1. Conformational changes

The seven peptides selected for study include the complete sequence of residues 24–199 of α_{s1}-casein, with some overlaps. Peptide 1–23 is the subject of a separate investigation comparing conformations predicted by NMR, CD, FTIR, and molecular modelling. No major differences have been observed between the conformations of peptide 1–23 refined while solvated (Malin et al., in preparation) and those of the peptide refined in vacuo (Malin & Brown, 1995). Structural features of the seven peptides before and after molecular dynamics and associated minimizations are outlined in Table 1. In Figs. 1–3, backbone representations of six peptides are used to compare their initial conformations, as excised from the refined model of α_{s1}-casein (Kumosinski et al., 1994a), and those predicted by molecular modelling as solvated peptides.

3.2. Peptide 24–101

The largest peptide investigated, residues 24–101, contains seven of the eight phosphoserine residues in α_{s1}-casein. The initial conformation of residues 24–101 as it appears in the complete refined α_{s1}-casein model is shown in Fig. 1. The conformation after solvation and molecular dynamics, Fig. 1b, exhibits significant changes in the regions 24–40 and 78–101 but relatively little change in the phosphoserine region, 46–75. A length of α-helix at the carboxyl terminal has developed a more complete loop, but the extended stretch from 78–89, identified as β-sheet like by the modelling program, has disappeared and is replaced by a twist.

To explore the resistance to conformational change in the phosphoserine region, the peptide was mutated on the computer by replacing phosphoserine residues with serine (Pse ⇒ Ser) and then refining the mutated peptide with molecular dynamics-energy minimization. The refined mutated peptide is shown in Fig. 1c. In its centre

[1] Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

Table 1
Effect of molecular dynamics on energies and structural features of α_{s1}-casein peptides

Peptide	No. res.	No. H_2O	Hydration, g H_2O/ g peptide	Energy, kcal/res/mol		C_α–C_α Distance, Å		α-Helix		β-Sheet		Turn	
				Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
24–101	78	343	0.65	1729.3	− 6535.6	23.9	17.2	1	1	1	0	7	3
24–101 (Pse ⇒ Ser)	78	328	0.66	1729.3	− 6165.5	23.9	16.0	1	1	1	0	7	9
91–101	11	49	0.64	51.7	− 579.8	14.7	12.8	1	1	0	0	1	0
102–142	41	146	0.55	441.8	− 3172.3	26.0	20.2	1	0	1	0	5	3
143–151	9	32	0.55	115.9	− 402.5	5.7	4.3	0	0	1	0	1	0
143–164	22	100	0.64	266.0	− 685.2	30.6	26.9	0	0	2	0	2	3
152–164	13	61	0.71	161.3	− 887.5	24.6	22.4	0	0	1	0	1	0
152–199	48	225	0.74	630.7	− 3148.1	55.3	52.6	0	0	3	0	6	5

Initial = as excised from the refined model of α_{s1}-casein (Kumosinski et al., 1994a); final = after molecular dynamics and minimizations.

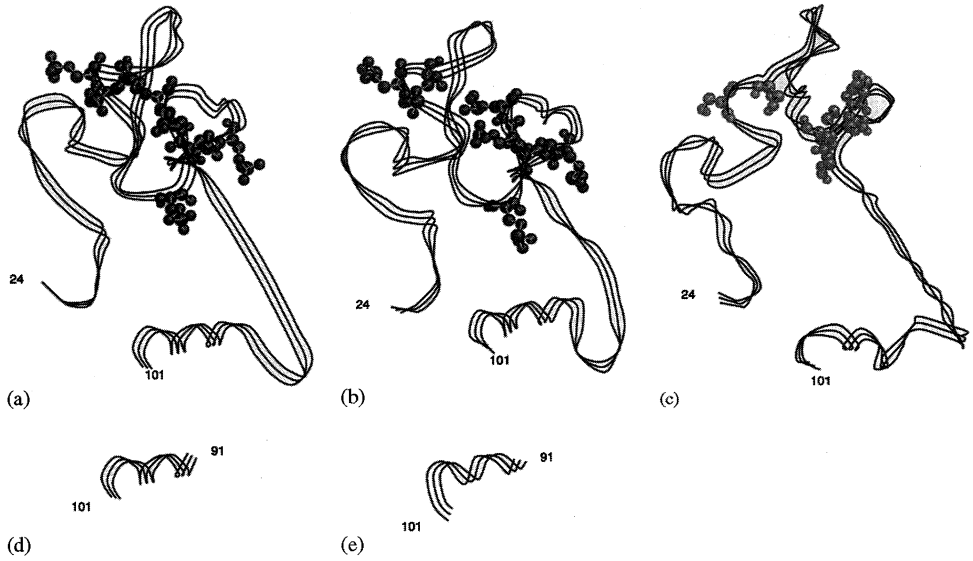

Fig. 1. Backbone conformations of peptides 24–101 and 91–101. Atoms of phosphoserine residues are shown as dark balls and atoms of serine replacements as grey balls. (a) Peptide 24–101 after excision from the intact model of α_{s1}-casein. (b) Peptide 24–101 after molecular dynamics simulations and energy minimizations. (c) Mutated peptide 24–101 in which phosphoserine residues were replaced by serine. (d) Peptide 91–101 after excision from the intact α_{s1}-casein model; conformation is identical to that of the corresponding residues in (a). (e). Peptide 91–101 after molecular dynamics simulations and energy minimizations; conformation differs from that of the corresponding residues in (b) and (c).

section some large turns have disappeared and are replaced by more numerous, smaller ones (Table 1). As changes occurred in the sequence 46–75 the overall conformation of the mutated peptide was destabilized, allowing the C-terminal helix to unfold somewhat and become distorted. Charged phosphoserine residues, therefore, prevent major changes in local conformation in residues 46–75, as repulsive forces maintain charge separation. Similar results were found by Kumosinski and Farrell (1994). Addition of calcium ions to the model may have the same effect as the mutation.

The effects of neighbouring residues on the conformational stability of the C-terminal portion of peptide 24–101 were examined in greater detail (Fig. 1d, e). Side chains of the N-terminal sequence (Phe_{24}–Val_{25}–Ala_{26}) are closer to those of 91–101 after refinement (Fig. 1b); this arrangement may provide a local helix-promoting environment resulting in formation of a third complete turn (Fig. 1b). The incomplete formation of the third helical turn when peptide 91–101 (Fig. 1d) is refined in the absence of the 24–90 sequence (Fig. 1e) supports this possibility.

3.3. *Peptide 102–142*

Although peptide 102–142 (Fig. 2) exhibited the greatest tendency to acquire a more compact conformation, some elements of secondary structure were not

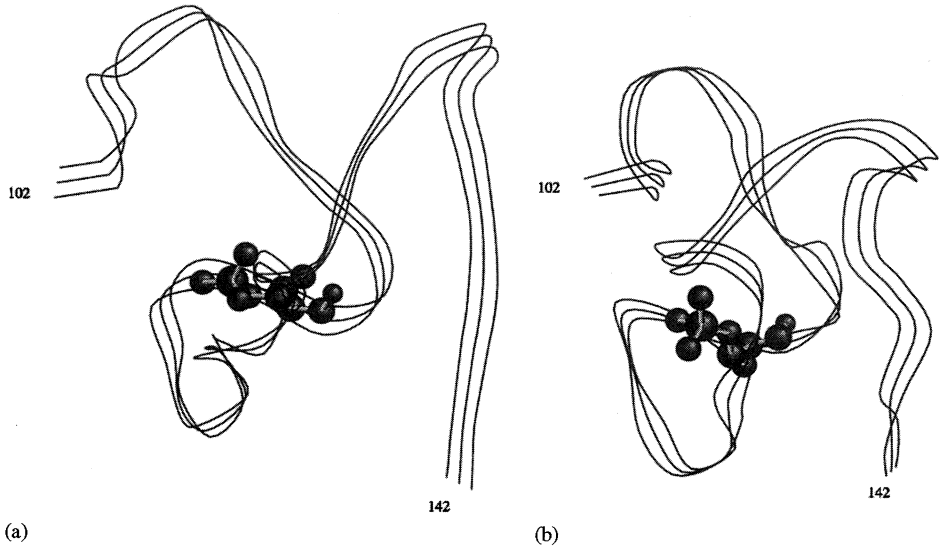

Fig. 2. Backbone representations of peptide 102–142. Atoms of phosphoserine shown as dark balls. (a) After excision from the intact α_{s1}-casein model. (b) After molecular dynamics simulations and energy minimizations.

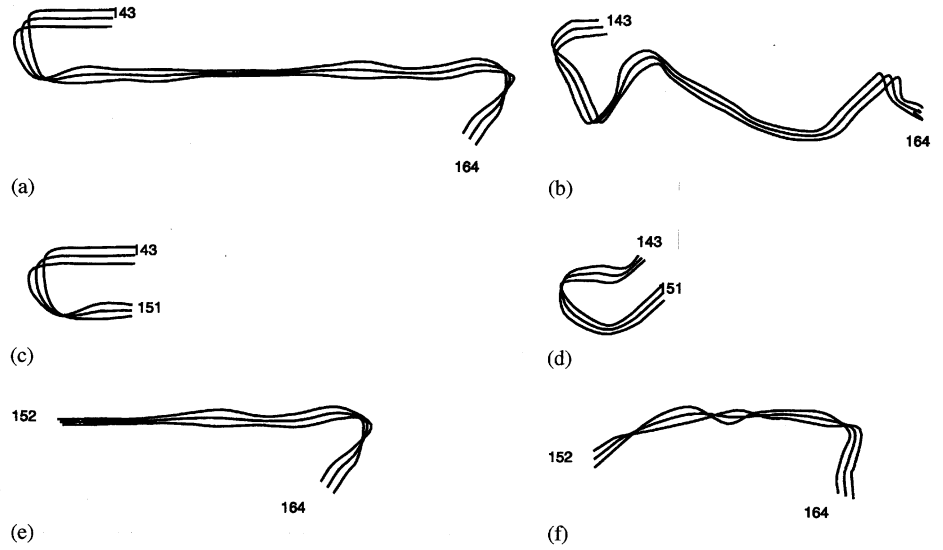

Fig. 3. Backbone representations of peptides 143–164, 143–151, and 152–164. (a) Peptide 143–164 after excision from the intact α_{s1}-casein model. (b) Peptide 143–164 after molecular dynamics simulations and energy minimizations. (c) Peptide 143–151 after excision from the intact α_{s1}-casein model; conformation is identical to that of the corresponding region in (a). (d) Peptide 143–151 after molecular dynamics simulations and energy minimizations; conformation differs from that of the corresponding region in (b). (e) Peptide 152–164 after excision from the intact α_{s1}-casein model; conformation is identical to that of the corresponding region in (a). (f) Peptide 152–164 after molecular dynamics simulations and energy minimizations; conformation differs from that of the corresponding region in (b).

retained in the refined structure. A single α-helical turn, 125–128, and two loops in the region 117–124 in the peptide as extracted from the α_{s1}-casein model (Fig. 2a) are not observed in the solvated peptide after refinement (Fig. 2b). Residues 117–124 form a slight curve, and the φ/ψ angles of residues 125–128 no longer meet the criteria of the modelling program for α-helix, forming a loop instead. In Fig. 2a, the sidechain of Pse_{115} is directed away from sidechains of the loops in the region 117–124; in Fig. 2b, however, the Pse_{115} sidechain is directed toward the latter sidechains. This orientation may disrupt the loop formation in 117–124, which in turn may alter the φ/ψ orientation of residues 125–128.

The extended sequence, 136–142, in Fig. 2a has developed partial turns in the refined structure of Fig. 2b. The loss of features defined as β-sheet by the modelling program suggests that their presence was influenced by elements of the entire α_{s1}-casein model. That model

(Kumosinski et al., 1994a) shows a section of β-sheet composed of anti-parallel strands, 136–145 and 151–155, separated by a turn involving Pro_{147}. Without the last three residues (143–145) and especially without stabilization by the opposite strand of the β-sheet (151–155), the sequence 136–142 can adopt the more flexible conformation shown in Fig. 2b. The number of turns in peptide 102–142 decreased after refinement (Table 1) from five to the three that include Pro_{107}, Pro_{113}, and Pro_{134}.

3.4. Peptide 143–164

The remainder of the anti-parallel β-sheet that begins with residues 136–142 (Fig. 2) is contained in the next peptide, 143–164 (Fig. 3). The initial conformation is shown in Fig. 3a, but after refinement only the turns remain. The extended conformation of residues 151–155 has changed into a curving structure (Fig. 3b), recognized as an additional turn by the modelling program (Table 1), probably because the opposite strand of the β-sheet (residues 136–142, Fig. 2a) is absent. Although residues 143–145 are part of this peptide, they do not provide sufficient stability to the extended structure of 151–155.

Further investigation of peptide 143–164 was focused first on peptide 143–151 (Fig. 3c, d). This sequence includes the turn that is an essential part of the anti-parallel β-sheet (136–145 and 151–155) in the complete α_{s1}-casein model (Kumosinski et al., 1994a). The hydrogen bonding pattern in the initial conformation of 143–151 (Fig. 3c) maintains the rigidity of the β-sheet. However, without the strands composing the β-sheet, hydrogen bonding is altered and residues 143–151 have enough flexibility to adopt lower energy conformations during refinement (Fig. 3d).

Peptide 152–164 (Fig. 3e, f) shows another aspect of peptide 143–164. In the initial conformation (Fig. 3e), residues 152–155 are part of the anti-parallel β-sheet (136–145 and 151–155). The absence of residues 143–151, discussed above, allows greater conformational flexibility, and residues 152–155 in the refined peptide (Fig. 3f) have developed a more greatly curved conformation than in 143–164. The turn that includes Pro_{160} is still present, but residues 163–164 have curved outward as well. These results suggest that residues 143–151 limit the folding patterns of the refined structure of 143–164; with 143–151 absent, residues 152–155 can adopt a more compact conformation.

3.5. Peptide 152–199

The final peptide, 152–199 (not shown), appeared to undergo relatively few changes during refinement. Three sections of extended β-sheet-like sequences (152–155, 164–166, and 170–174) are identified by the modelling program (Table 1) in the initial peptide; the latter two constitute an anti-parallel β-sheet. Residues 152–155 are not extended in the refined peptide; the β-sheet is distorted and, therefore, no longer recognized by the modelling program, which bases identification on φ/ψ angles. Other sections of the peptide have also adopted more flexible conformations. Although there seem to be a greater number of turns, they are not recognized by the modelling program because φ/ψ angles of the involved residues are not within the limits defined by the SYBYL program.

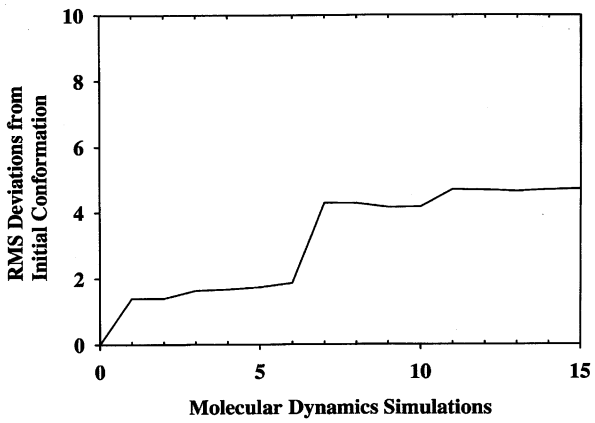

Fig. 4. Conformation changes in peptide 102–142 during refinement by molecular dynamics simulations and energy minimizations, as measured by RMS deviations in backbone conformation from that of the initial peptide as excised from the intact α_{s1}-casein model.

3.6. Changes in physical attributes

A fitting algorithm was used to characterize the progress of refinement for a typical peptide, 102–142. The root-mean-square (RMS) deviations of the main chain (backbone) conformation from the initial excised sequence (Fig. 2a) were evaluated after each session of molecular dynamics simulation and energy minimization. Fig. 4 shows the gradual increase in RMS deviations as the peptide adopted new conformations along the pathway to a lower negative energy indicative of a new stable structure (Table 1). Energies of peptide 102–142 gradually decreased from 441.8 to −3172.3 as the RMS deviations increased (Table 1).

4. Discussion

An important tenet of protein structure (Anfinsen, 1973) is that the amino acid sequence of a protein contains all the information needed to determine its most accessible stable structure. While this is an accepted rule for all proteins, its proof has been demonstrated most often in typical globular proteins. The results of the molecular dynamics simulations presented here show that peptides of a protein with a more open structure can acquire new stable conformations when released from

spatial constraints imposed by the intact parent protein. The driving force for this process is the search for large, negative free energies which are characteristic of any stable, folded protein or peptide. The role of molecular dynamics in providing energy for the conformational changes in a hypothetical peptide is analogous to placing a real peptide in a solution of optimum pH and ionic strength in which it can fold naturally. New conformations will be adopted in real casein peptides released by the action of agents such as enzymes, whether in milk, cheese, or any other dairy product. The nature of these conformational changes will depend on the extent of interactions with neighbouring peptides and proteins.

In some peptides, conformations were altered significantly after refinement by molecular dynamics and energy minimizations—102–142, for example—whereas only small changes were observed in others, such as 152–199. In all peptides investigated (Table 1), the C_α–C_α distance decreased between the amino and carboxyl termini of the peptide. This is indicative of more compact conformations and is a characteristic of folded proteins (Christopher & Baldwin, 1996).

Elements of secondary structure, such as α-helix and β-sheet, frequently disappeared during refinement in the α_{s1}-casein peptides. Extended β-sheet conformations in these peptides are usually part of hydrogen-bonded β-sheets in the intact model and are, therefore, stabilized by the structure of the intact model. In the two α-helices investigated, residues 91–99 in peptide 24–101 remain helical during refinement of the native sequence but are replaced by a single helical turn, residues 94–96, in the mutated peptide in which serines replaced phosphoserine residues. In peptide 102–142, the helical turn of residues 125–128 becomes a loop, possibly because of the effect of Pse_{115} on residues 117–124. These results suggest that the surrounding intact protein has a smaller influence on helical conformations than on extended β-sheet-like structures. Turns generally were retained during refinement, also suggesting a greater influence of sequence than of local environment. The large number of proline residues in caseins prevents the formation of significant amounts of helix, promotes the formation of β-sheet, and is largely responsible for the open, extended structure required for functional properties.

In summary, sequences in α_{s1}-casein peptides that promote the open, flexible structure in the parent protein are likely to resist formation of new identifiable secondary structure during refinement but are also capable of becoming more flexible and developing turns, a consequence of the high proline content. When the sidechains of specific residues prevent formation of new conformations, as in residues 46–75 of peptide 24–101, because of the charged phosphate groups on the sidechains, addition of Ca^{2+} to the model should overcome resistance to the refinement process. This possibility is currently under investigation.

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